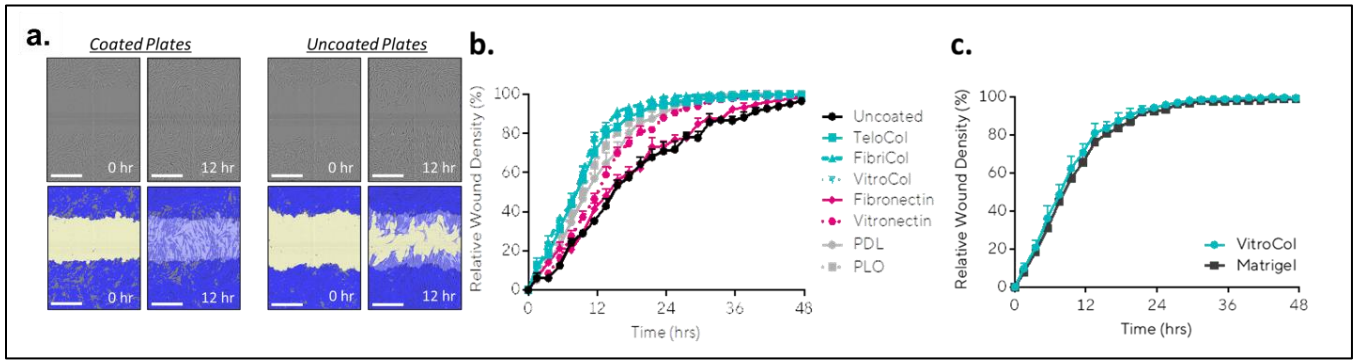
Xeno-Free ECM Coatings Promote Fibroblast Migration and Wound Healing

Fibroblast migration is critical in wound healing. This study demonstrates the superior performance of Advanced BioMatrix's xeno-free extracellular matrix (ECM) coatings in promoting fibroblast migration using the Scratch Wound Assay. The xeno-free coatings showed faster migration and consistent wound closure compared to uncoated plates and Matrigel®, offering a physiologically relevant and reproducible platform for migration assays.

Materials	Catalog Number	Final concentration
VitroCol® (xeno-free)	5007	100 µg/mL
Fibronectin (xeno-free)	5050	100 µg/mL
Vitronectin (xeno-free)	5051	100 µg/mL
Poly-D-Lysine (xeno-free)	5049	100 µg/mL
Poly-L-Ornithine (xeno-free)	5058	100 µg/mL
FibriCol®	5133	100 µg/mL
TeloCol-3®	5026	100 µg/mL

Advanced BioMatrix's xeno-free ECM coatings, particularly VitroCol®, support faster and consistent fibroblast migration. These coatings offer a biologically relevant, contamination-free alternative to traditional matrices, advancing wound healing research and regenerative medicine.



Fibroblast migration on coated and uncoated plates. Xeno-free VitroCol® demonstrated similar cell migration rates to Matrigel® control, suggesting its capability in promoting migration and wound healing.

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Abstract

The scratch wound assay is a widely used method to study cellular migration, a critical aspect of wound healing. This white paper investigates the impact of xeno-free extracellular matrix (ECM) coatings on fibroblast migration using the Incucyte® Scratch Wound Assay. The Advanced BioMatrix (ABM) xeno-free ECM coatings demonstrated superior migration rates and consistent wound closure compared to uncoated plates and with Matrigel®. This study highlights the potential of xeno-free ECM solutions to improve the physiological relevance and reproducibility of migration assays.

Table 1. Materials used to evaluate fibroblast wound healing capability.

Materials	Supplier	Cat. Number	Final concentration
VitroCol® (xeno-free)	Advanced BioMatrix	5007	100 µg/mL
Fibronectin (xeno-free)	Advanced BioMatrix	5050	100 µg/mL
Vitronectin (xeno-free)	Advanced BioMatrix	5051	100 µg/mL
Poly-D-Lysine (xeno-free)	Advanced BioMatrix	5049	100 µg/mL
Poly-L-Ornithine (xeno-free)	Advanced BioMatrix	5058	100 µg/mL
FibriCol®	Advanced BioMatrix	5133	100 µg/mL
TeloCol-3®	Advanced BioMatrix	5026	100 µg/mL
Matrigel®	Corning	354234	100 µg/mL

Introduction

Fibroblast migration is a key process during the proliferative phase of wound healing, where cells traverse the extracellular matrix (ECM) to reconstruct damaged tissue.[1,2] Traditional in vitro models often rely on animal-derived ECM components, which introduce contaminants and immunological concerns. To address these limitations, xeno-free ECM coatings provide a biologically relevant alternative.[3,4]

This study evaluates the performance of several Advanced BioMatrix's biomaterials to support fibroblast migration and wound healing. These include a xeno-free collagen I solution, VitroCol®, Fibronectin, Vitronectin, Poly-D-Lysine (PDL), Poly-L-Ornithine (PLO) as well as TeloCol-3® and FibriCol®. The scratch wound assay, combined with live-cell imaging using the Incucyte® system, was employed to quantify cell migration dynamics.

Results

TeloCol®, FibriCol®, VitroCol®, Fibronectin, Vitronectin, PDL, and PLO from Advanced BioMatrix (ABM) were characterized for their ability to support wound healing for NHDFs. Phase images of NHDFs on coated plates showed clear, high-quality images, free of background noise and successful attachment comparable to uncoated plates (Fig 2A) at 0 hours. In addition, the coated-plates demonstrated improved wounding of cells using the Incucyte® 96-Well Woundmaker Tool, creating more consistent and straighter scratches compared to uncoated plates. Cellular masking using Incucyte® Live Cell Analysis was also preserved and comparable between conditions. After 12 hours, the NHDFs were able to successfully migrate and close the wound on coated plates, while the wound was still present for the uncoated condition. Quantification of the relative wound density over time confirmed these observations, demonstrating faster migration and wound closure over uncoated plates for most ABM solutions, except for fibronectin (Fig 2B). Furthermore, the collagen solutions showed the fastest migration, suggesting an optimal coating solution for NHDFs. Additional experiments were performed comparing ABM's xeno-free collagen I solution, VitroCol®, to Matrigel®, and the results (Fig 2C) demonstrated similar migration rate, suggesting VitroCol® as an alternative matrix to Matrigel® for migrations assays.

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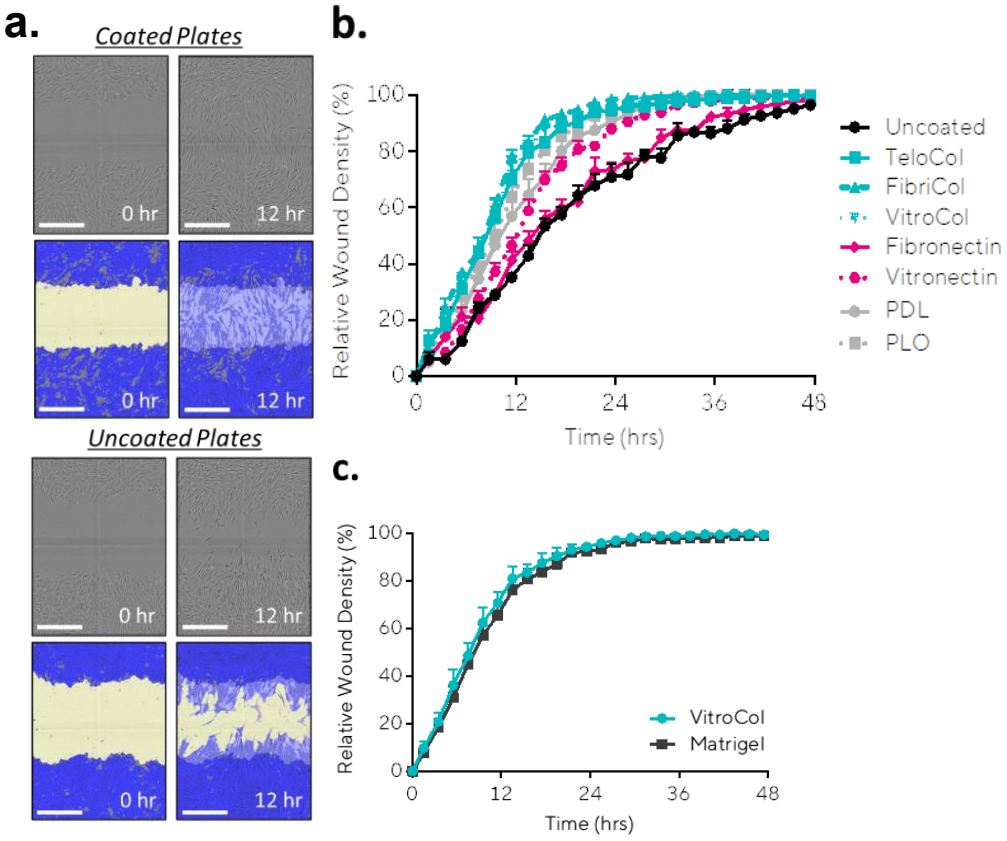

Figure 1. Advanced BioMatrix coatings support cellular migration for wound healing. NHDFs were seeded at 15K cells/well on collagen-coated or uncoated 96-well plates and incubated for 24 hours to adhere, before wounding using an Incucyte 96-well Woundmaker Tool. Cellular migration was visualized by HD phase microscopy using an Incucyte SX5 Live-Cell Analysis System with masked cells (dark blue), initial scratch (light blue), and wound closure (yellow) monitored over time. Scale bars = 500 µm. The relative wound density was quantified over time for (B) seven different ABM coated plates against uncoated cell culture plates and (C) xeno-free VitroCol® collagen-I coated plates against Matrigel coated plates (data represents mean ± SD).

Conclusion

This study demonstrates the superior performance of ABM coating biomaterials, especially the xeno-free VitroCol® ECM coatings, in supporting fibroblast migration and wound healing. The coatings matched the efficacy of Matrigel® while eliminating the risks associated with animal-derived contaminants. By enhancing wound closure and providing a physiologically relevant

platform, the xeno-free ECM coatings offer significant advantages for wound healing research, drug development, and regenerative medicine.

Materials and Methods

Coating Preparation

VitroCol®, TeloCol-3®, FibriCol®, Fibronectin, Vitronectin, PDL, or PLO from Advanced BioMatrix, as well as the Matrigel control (Table 1) were added at a concentration of 100 µg/mL to Incucyte® Imagelock 96-well Plates (Satorius, Cat. No. 4806). Each solution was added at 50 µL to each well and the plate was air-dried in a biosafety cabinet for 2 hours at room temperature to produce the monolayer coatings. After drying, the coatings were rinsed with DPBS once and dried completely before cell culture.

Cell Culture

Normal human dermal fibroblasts (NHDFs) were cultured at 37°C and 5% CO_2 in Dulbecco's Modified Eagle Media (DMEM) with 10% fetal bovine serum (FBS). Cells were cultured to 90-95% confluency and then passaged using TrypLE™ into a new flask at ~7000 cells/cm^2. Culture media was replaced every 72 hours, and cells from passage 4–8 were used in experiments.

Scratch Wound Migration Assay

NHDFs were harvested using TrypLE and resuspended in fresh DMEM with 10% FBS. NHDFs were counted and added to the coated plates at a density of 15,000 cells/well. The plates were placed in an incubator overnight to allow cell adhesion. In the next morning, the plate lid was removed and an Incucyte® 96-Well Woundmaker Tool (Satorius, Cat. No. 4563) was used to simultaneously create wounds in all wells. After wounding, the media was aspirated, and cells were washed twice with 100 µL of culture media. Then, 100 µL of fresh media was added to each well before placing in an Incucyte® SX5 Live Cell Analysis System for image acquisition and analysis (Fig 2).

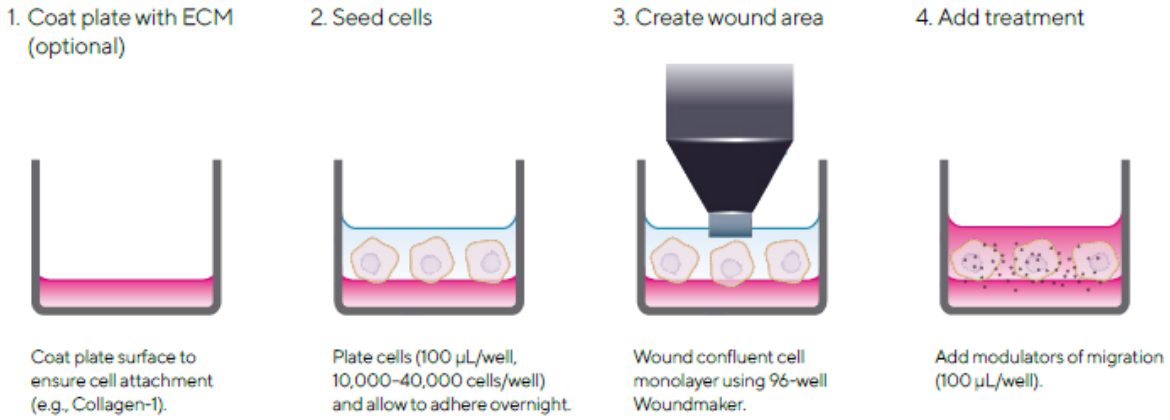

Figure 2. Schematic of Scratch Wound Migration Assay

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